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Biomarkers of β-Cell Stress and Death in Type 1 Diabetes

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Abstract

The hallmark of type 1 diabetes (T1D) is a decline in functional β-cell mass arising as a result of autoimmunity. Immunomodulatory interventions at disease onset have resulted in partial stabilization of β-cell function, but full recovery of insulin secretion has remained elusive. Revised efforts have focused on disease prevention through interventions administered at earlier disease stages. To support this paradigm, there is a parallel effort ongoing to identify circulating biomarkers that have the potential to identify stress and death of the islet β-cells. Whereas no definitive biomarker(s) have been fully validated, several approaches hold promise that T1D can be reliably identified in the pre-symptomatic phase, such that either β-cell preservation or immunomodulatory agents might be employed in at-risk populations. This review summarizes the most promising protein- and nucleic acid-based biomarkers discovered to date and reviews the context in which they have been studied.

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Compliance with Ethical Standards

Conflict of Interest Raghavendra G. Mirmira, Emily K. Sims, Farooq Syed, and Carmella Evans-Molina declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Keywords

Type 1 diabetes; miRNA; Cell-free insulin DNA; Biomarkers; Unmethylated insulin DNA; Proinsulin; Proinsulin/C-peptide ratio

Introduction

Type 1 diabetes (T1D) is characterized by the immune-mediated destruction of the insulin producing pancreatic β-cells. T1D arises from a break in immune tolerance and infiltration of auto-reactive T cells that target the β-cells, leading to loss of β-cell function and mass and a lifelong requirement for exogenous insulin [1]. To preserve β-cell function, a number of immunomodulatory drugs have been tested around the time of T1D clinical diagnosis. A handful of drugs, including anti-CD3, anti-CD20, CTLA4-Ig, and alefacept have led to a modest preservation of β-cell function [2–6]. However, true remission from T1D, as defined by insulin independence, remains elusive. These outcomes could be explained by the low proliferative and regenerative capacity of human β-cells [7, 8], combined with the possibility that interventions were initiated too late in disease, occurring after the time in which intrinsic stress pathways in β-cells have been so robustly activated that even modulation of autoimmunity could not prevent β-cell decline, or treating autoimmunity with the depleting/immunomodulatory agents mentioned above does not address the coexisting β-cell dysfunction. This may be an equally important pathogenic abnormality that should be also targeted therapeutically. In recent years, data characterizing intrinsic β-cell stress in T1D suggest that processes such as β-cell calcium dyshomeostasis, misfolded protein accumulation, oxidative stress, and endoplasmic reticulum stress become activated early during the evolution of T1D and act to either initiate immune activation through formation of neoantigens or serve to augment autoimmune-mediated β-cell death and dysfunction [9–11]. Whereas these pathways may serve as points of therapeutic intervention, focused methods to identify biomarkers that reflect an authentic signature of β-cell stress and/or death may also improve our ability to optimally time immunomodulatory and other forms of interventions in T1D. In such a paradigm, a valid signature of β-cell stress could serve as a signal to initiate immunomodulatory therapy in the pre-T1D diabetic period. Thus, interventions in the newly defined stages 1–2 of the pre-symptomatic phase of T1D [12] offer the promise of more effective preservation of β-cell mass. However, a challenge in the field of β-cell biomarkers is the fact that β-cells represent a very small fraction (≤1 %) [13] of total pancreatic mass and an even smaller fraction of total body cellular mass. Historically, detection of biomarkers that emanate from the β-cell has been largely outside of the technical capacity of most laboratories. However, with the advent of next-generation sequencing technologies and more sensitive/specific nucleic amplification, mass spectroscopic, and antibody-based methodologies, both the identification and detection of promising biomarkers that are β-cell specific/selective is becoming a reality. Here, we review the status of select protein and nucleic acid biomarkers that provide insight into the health of the β-cell and have the potential, alone or in combination, to identify individuals with T1D who may benefit from therapies aimed at preservation of insulin secretion.

Proinsulin/C-peptide Ratio as a Circulating Biomarker of β-Cell Stress in T1D

The islet β-cell is a highly secretory cell that is singularly tasked with the heavy biosynthetic burden of insulin production. Efficient protein translation and processing requires a robust endoplasmic reticulum (ER). Under conditions of T1D autoimmunity and inflammatory stress, insulin demand may exceed the ability of the β-cell ER to process newly translated proteins, leading to accumulation of unfolded proteins. If this process continues without resolution, it will lead to activation of ER stress and pro-apoptotic signaling pathways [14]. A growing number of preclinical studies suggest that β-cell ER stress contributes to the pathogenesis of T1D, prior to the onset of hyperglycemia [15–17]. In human T1D, immunostaining of islets from donors with T1D revealed abnormal expression of ER stress markers, including C/EBP homologous protein (CHOP), activating transcription factor 6 (ATF6), and spliced X-box binding protein 1 [15, 18].

Insulin biosynthesis begins with the production of preproinsulin at ribosomes. Preproinsulin is then converted to proinsulin within the ER by cleavage of the N-terminal signal peptide. Further processing and cleavage occurs in the ER, Golgi network, and secretory granules before release of mature insulin and C-peptide into the circulation [19]. A hallmark of β-cell ER dysfunction is the accumulation and secretion of inadequately processed proinsulin molecules [14]. Therefore, β-cell ER stress may be detectable in a minimally invasive manner via measurement of the ratio of circulating proinsulin relative to insulin or C-peptide [16]. Using proinsulin/C-peptide (PI/C) ratios as a non-invasive marker of β-cell dysfunction could provide a means to identify individuals at risk of developing T1D prior to the onset of massive β-cell destruction. Evaluation of this ratio may also provide a means to monitor disease evolution after diagnosis [20]. In considering studies that examined PI/C ratios (see Table 1), it is important to recognize that ELISAs for proinsulin and C-peptide have evolved considerably over the last several years. Current assays exhibit significantly greater sensitivity and specificity.

A series of studies in Northern Europe have examined PI/C ratios in family members at risk for T1D. Fasting PI/C ratios were inversely correlated to first phase insulin response during an intravenous glucose tolerance test performed on 23 antibody-positive Finnish siblings of persons with T1D [25]. Among siblings followed longitudinally, 7/9 who progressed to develop T1D had an increase in PI/C ratio during the 6 months prior to diagnosis [39]. In the Belgian Diabetes Registry, autoantibody-positive first-degree relatives with random PI/C ratios above the 66th percentile had an increased 5-year risk of T1D development [26]. Sims et al. recently reported that within the TrialNet Pathway to Prevention cohort, an international cohort of autoantibody-positive family members of individuals with T1D, fasting PI/C ratios were significantly increased ~12 months prior to onset of T1D [27••]. Elevations were most pronounced in children ≤10 years, where median PI/C ratios were 3-fold higher than those who did not progress to T1D. However, even among the entire group, increased PI/C ratios were associated with increased odds of progression to T1D, after correction for age and BMI [27••].

Several groups have identified on average a 3–10-fold elevation in PI/C ratios at T1D diagnosis compared to controls [21–23]. Although C-peptide levels are typically increased in the months after diagnosis, this period has still been largely associated with a concomitant increase in proinsulin levels, with varying patterns in PI/C ratios during this partial remission or honeymoon period [21–24]. PI/C ratios at diagnosis are inversely correlated with length of the honeymoon period [21]. Treatment of new-onset subjects with cyclosporine reduced PI/C ratios, with an increased percentage of subjects experiencing a non-insulin requiring honeymoon [22]. Data regarding PI/C ratios in longer-standing T1D is more limited. Among 73 Swedish pediatric subjects with T1D for >2 years, fasting proinsulin and C-peptide levels were highly correlated. Notably in that study, proinsulin was still detected in 27.8 % of subjects with a negative fasting C-peptide [40], suggesting either complete failure to process proinsulin in these individuals or significant differences in sensitivity of the assays for proinsulin and C-peptide.

Intriguingly, proinsulin/C-peptide ratios also appear to be increased in lower risk family members compared to controls with no family history of type 1 diabetes. Several cohorts have identified elevations in fasting proinsulin despite similar fasting C-peptide/insulin or blood glucose levels in siblings of subjects with type 1 diabetes, independent of high-risk HLA status [41–43]. Increased fasting proinsulin levels have also been reported in parents and children of individuals with type 1 diabetes [43]. Small-scale studies have shown elevations in fasting proinsulin, despite similar C-peptide values, in unaffected monozygotic twin siblings. Elevated proinsulin levels were independent of islet cell antibody positivity and were present >10 years after the sibling's diabetes diagnosis [44, 45].

These findings could represent an inherited predisposition to β-cell dysfunction that could theoretically contribute to type 1 diabetes pathogenesis in affected families. Alternatively, increased ratios could reflect a history of autoimmune β-cell injury that did not progress to frank diabetes. Interestingly, analysis of random proinsulin/C-peptide ratios in cord blood of newborn siblings of affected patients showed no difference compared to controls [46]. These findings may point to later timing of an inherited β-cell defect versus physiologic differences in newborn proinsulin processing that masks familial disparities. Notwithstanding these questions, PI/C ratios remain likely to be an informative and useful component of T1D risk prediction algorithms, as well as an important biomarker of β-cell dysfunction in T1D treatment trials. Moreover, additional studies are needed to understand better the molecular mechanisms underlying these abnormalities.

Non-coding RNAs as Circulating Biomarkers of β-Cell Stress in T1D

Non-coding RNAs comprise a large group of RNA molecules that function post-transcriptionally to regulate a host of cellular activities ranging from differentiation and proliferation to apoptosis. They include classes of RNAs referred to as micro RNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), small nucleolar RNAs (snoRNAs), and stable intronic sequence RNAs (sisRNAs) among others. The best characterized class of non-coding RNAs with respect to β-cell function and biomarker development is the miRNAs. MicroRNAs are small RNA molecules of 21–23 nucleotides that post-transcriptionally regulate gene expression through either the inhibition of mRNA

translation or reduction of mRNA stability [47, 48]. In unusual cases, miRNAs can also positively regulate gene expression [49, 50]. In humans, over 2500 miRNAs have been cataloged [51], and it is estimated that collectively these miRNAs regulate approximately 60 % of protein-coding genes [52]. Whereas miRNAs are generated intracellularly and largely function in a cell-autonomous fashion, in recent years it has been appreciated that they can be selectively secreted extracellularly in microvesicles or exosomes and thereby transmit their regulatory functions to other cell types [53–55]. MicroRNAs have accordingly been regarded as potential circulating biomarkers that reflect activities of their cells of origin. In the context of diabetes, an emerging literature suggests that specific miRNAs or groups of miRNAs could serve as biomarkers of disease progression or diabetic complications [56–61]. The systemic stability of miRNAs relative to other RNA species allows for their measurement in a variety of biological fluids, including blood, urine, saliva, cerebrospinal fluid, milk, seminal fluid, and amniotic fluid, making them prime targets for biomarker discovery [62].

Because miRNAs are expressed broadly in a variety of tissues, none to date can be considered as exclusive to β-cells. However, several miRNAs have been proposed as biomarkers in individuals with T1D (see Table 1 for a summary). In-depth sequencing of human islets and isolated β-cells revealed >25 miRNAs that show relative enrichment in β-cells [63]. One of the most abundant miRNAs in β-cells is miR-375. MicroRNA-375 was identified early in studies of the mouse-derived β-cell line MIN6, where it was demonstrated to inhibit glucose-stimulated insulin owing to effects on vesicle exocytosis [64]. Using knockout mouse models, Poy et al. showed that miR-375 was required for the maintenance of normal β- and α-cell mass during mouse pancreas development [65]. The relative abundance and functional role of this miRNA naturally led to studies of its utility to serve as a biomarker of β-cell stress and/or mass. Erener et al. [28] showed in mice that streptozotocin (STZ) treatment, which selectively destroys β-cells, results in increases in circulating miR-375 levels, and that the NOD mouse model of T1D exhibited elevations in miR-375 during the weeks prior to development of frank hyperglycemia. In the islet transplantation setting in humans where acute β-cell death is observed, Kanak et al. [29••] showed that circulating miR-375 levels were elevated immediately post-transplantation and remained elevated up to 7 days later. In the T1D setting, C-peptide-negative individuals exhibited higher circulating miR-375 levels compared to individuals with more substantial β-cell mass, such as non-diabetic controls, individuals with T2D, and individuals with maturity onset diabetes of the young (MODY) [66••]. These findings suggest that steady-state circulating levels of miR-375 are more likely a reflection of dying β-cells rather than prevailing β-cell mass.

To identify other miRNAs that contribute to the pathogenesis of T1D and might serve as biomarkers of β-cell stress, Roggli et al. [30] performed global microarray profiling of human islets treated with a cocktail of proinflammatory cytokines and demonstrated increases in miR-21, miR-34a, and miR-146a. Notably, these same miRNAs were elevated in the islets of pre-diabetic NOD mice, and blockade of these miRNAs using anti-miRs prevented the loss in insulin secretion from MIN6 β-cells exposed to proinflammatory cytokines [30]. Using a different stress paradigm of T1D, Kim and colleagues recently demonstrated increases in miR-34a-5p, miR-21-3p, miR155-5p, miR-1290, miR663b, and

miR10b-3p in human islets infected with Coxsackievirus B5, and bioinformatics analysis identified 57 candidate type 1 diabetes risk genes predicted to be direct targets of Coxsackievirus B5 responsive miRNAs [32].

These findings suggested a pathogenic role of certain miRs in β-cell dysfunction in T1D, but still did not address whether these and other miRs might serve as circulating biomarkers reflecting β-cell stress. To address this possibility, Nielsen et al. [31] undertook a comprehensive sequencing analysis using serum of subjects with new-onset T1D. Relative to control subjects after adjustment for age and gender, 12 upregulated miRNAs were identified in the serum of new-onset T1D subjects, including miR-152, miR-30a-5p, miR-181a, miR-24, miR-148a, miR-210, miR-27a, miR-29a, miR-26a, miR-27b, miR-25, and miR-200a [31]. Other studies have identified additional miRNAs associated with T1D, emphasizing the correlation between miRNAs and T1D diagnosis and/or control [67, 68]. Notably, whereas some of the miRNAs have been shown to be enriched in human β-cells (e.g., miR21, miR24, miR29a, miR375) [63], many are not, suggesting that they may not be a reflection of endogenous β-cell stress. Nevertheless, miRNAs represent a potentially promising class of circulating nucleic acids that could serve as biomarkers of individuals at high-risk for T1D (Table 1), and future studies in high-risk populations are warranted.

Data on the role and potential as biomarkers of other non-coding RNAs are limited. An emerging class with biomarker potential is the lncRNAs. LncRNAs are defined as non-coding RNAs of >200 nucleotides in length. Although the mechanisms of lncRNAs is not well understood, like miRNAs, lncRNAs appear to function in a diversity of cellular processes, such as development and differentiation, proliferation, and apoptosis (for a recent review of lncRNAs in autoimmune diseases, see [69]). According to the NONCODE database [70], more than 140,000 lncRNA transcripts (encoded by more than 90,000 genes) have been identified in humans. As such, the potential for these RNAs to serve as biomarkers of disease susceptibility and development is gaining traction. Based on genome-wide association studies, several lncRNAs have been associated with T1D susceptibility [71–74]. To date, however, studies are limited with respect to the expression and function of lncRNAs in the β-cell. A recent study in mouse β-cell-derived cell lines and NOD mouse islets showed that lncRNAs 1-4 are strongly upregulated in response to proinflammatory cytokines and disease progression, respectively [75]. Carter et al. [76] showed that in humans, the lncRNA GAS5 was negatively correlated with the prevalence of type 2 diabetes in a Veteran's Administration cohort. Thus, further studies on the potential for lncRNAs and other non-coding RNAs to serve as circulating biomarkers of β-cell stress is warranted.

Differentially Methylated DNA as a Biomarker of β-Cell Stress and Death

The presence of circulating, cell-free nucleic acids derived from dying cells has been recognized for over 60 years, and more recently the detection and quantitation of such nucleic acids has been used to detect apoptosis/necrosis and/or turnover of their cells of origin (for a review, see [77]). One particularly promising approach in biomarker development has been the detection of differentially methylated DNA fragments. DNA methylation at CpG sites occurs via the action of DNA methyltransferases in a chemical reaction involving cytosine and the cofactor S-adenosyl-L-methionine. In general,

methylation patterns at a given gene tend to be inversely correlated with activity of the gene. Thus, genes that are predominantly methylated at CpG dinucleotides are highly expressed, whereas genes that are predominantly unmethylated at these same dinucleotides tend to be quiescent. DNA methylation is therefore a key epigenetic component in many stably repressed loci (such as the inactivated X chromosome) throughout the genome and in specific cell types [78]. Just as importantly, dynamic changes to DNA methylation frequently account for malignant transformation of many cell types as a result of activation or inactivation of tumor-promoting or repressing genes/loci [79]. It was this link between DNA methylation status and tumor formation that led investigators to consider the possibility that circulating differentially methylated DNA fragments arising from transformed cells could serve as a biomarker for cancer [80].

The islet β-cell expresses multiple genes in a nearly exclusive manner, which accordingly have the potential to be regulated by selective DNA methylation. Perhaps the most abundant and exclusive gene is the one encoding preproinsulin (*INS* in humans and *Ins1* and *Ins2* in mice). Relative to multiple other cell types, the human and mouse genes have been shown to be hypomethylated in islets and β-cells at selective CpG sites in the promoter and coding regions [33, 35, 81], and human *INS* expression is inversely correlated with methylation at many of these sites in human islets [82]. As with cancer cells, the concept that cell-free, hypomethylated *INS* (or mouse *Ins1/Ins2*) appearing in the circulation might reflect an increase in β-cell death or turnover was first tested in early studies of Akirav et al. [35] using a SYBR Green dye-based methylation-specific PCR assay against a CpG site appearing in the coding region of the *Ins1* gene (at position +177 relative to the transcriptional start site). This study heralded the first generation of methylation-specific PCR assays in the T1D field, and involved a nested PCR approach, wherein isolated DNA from serum or plasma was subjected to bisulfite conversion, followed by a methylation insensitive PCR amplification step, manual extraction of the PCR product from a gel, followed by a methylation-specific PCR second step. The study by Akirav et al. [35] revealed that increases in circulating unmethylated mouse *Ins1* DNA (relative to the methylated counterpart) were detectable following STZ treatment of mice and in NOD mice prior to the onset of diabetes. Since the description of this first study, a subsequent study by Husseiny et al. [83] described the use of a similar SYBR Green-based assay for the mouse *Ins2* gene [83]. Other studies applied the assay to the analysis of human serum/plasma, showing that unmethylated *INS* (relative to methylated *INS* at the same CpG site) was increased at the time of T1D diagnosis [84] and following allogeneic islet transplantation [33]. Collectively, these studies provided the first evidence that β-cell-derived, unmethylated *INS* DNA correlates with the occurrence of clinical states where β-cell death is known to occur (see Table 1 for a summary of these studies).

The first generation assays were largely limited by the nature of SYBR Green-based methodologies, which included potential off-target annealing of methylation-specific primers, limited sensitivity that frequently required nested PCR strategies, and the requirement to perform separate PCRs for methylated and unmethylated target sequences. Fisher et al. [85] introduced a dual probe-based strategy (TaqMan), which allowed for the multiplex detection of both unmethylated and methylated *Ins2* DNA fragments (at position −182 bp relative to the transcriptional start site) in sera of mouse models of T1D, with low

background signals and without the need for nested PCR. Use of this dual probe-based technology signaled the development of second generation assays that engage droplet digital PCR (ddPCR), a technique that involves a microfluidics approach to generate thousands of droplets that undergo thermal cycling and utilizes Poisson statistics to obtain absolute copy numbers of DNA fragments [36••, 86]. Using ddPCR and a probe that simultaneously detects two methylation-sensitive sites of the human *INS* gene (at positions +396 and +399 relative to the transcriptional start site), Herold and colleagues [37••] showed that individuals at risk for T1D (autoantibody-positive individuals) who later progressed to T1D in the TrialNet Pathway to Prevention study collectively (averaged over several visits) had a statistically higher ratio of unmethylated/methylated *INS* DNA compared to healthy control subjects.

In a separate study utilizing ddPCR, Fisher et al. [36••] developed an assay targeting a single methylation-sensitive site at position −69 bp relative to the *INS* transcriptional start site, and showed that pediatric subjects with new-onset T1D (within 48 h of diagnosis) had higher absolute levels of both methylated and unmethylated insulin DNA compared to age-, gender-, and weight-matched non-diabetic controls. At 8 weeks post-diagnosis, the same individuals exhibited reductions in unmethylated *INS* DNA (to levels comparable to controls), but had persistent elevations in the methylated *INS* DNA levels. At 1 year post-diagnosis, both *INS* DNA species were at levels comparable to controls. These findings point to an important distinction between the ddPCR assays of Herold et al. [37••] and Fisher et al. [36••]. The former group uses ratios of unmethylated/methylated *INS* DNA levels, the latter examined absolute levels of each species separately. Whereas the unmethylated *INS* DNA is thought to arise predominantly from dying β-cells, the source of the elevated methylated *INS* remains unclear. Notably, Yang et al. [82] demonstrated that islets from subjects with type 2 diabetes showed greater methylation at several CpG sites compared to islets from control subjects, suggesting that β-cells might exhibit increasing methylation of the *INS* gene with progression of disease states. In agreement with this observation, a recent report by the Herold group [87•] showed that the methylation status of the *Ins1/Ins2* gene in the β-cell becomes more methylated with disease progression in the NOD mouse.

Because some non-β-cell types show low, but measurable frequency of unmethylated CpG sites in the *INS* gene [34••, 81], the aforementioned studies cannot rule out the possibility that the signals corresponding to unmethylated *INS* DNA might be arising (in part) from non-β-cells. To address this concern, the studies of Lehmann-Werman et al. [34••] used next-generation sequencing methods to simultaneously detect methylation at six distinct CpG sites in the *INS* promoter to achieve very high specificity (since all six sites are simultaneously unmethylated in fewer than 0.01 % of DNA molecules arising from non-β-cells). Using this approach, these authors showed that they were able to distinguish subjects with recent-onset T1D from healthy controls in a small cohort with 100 % sensitivity and specificity. As noted above, however, further studies will be needed in larger cohorts to know whether dynamic methylation at CpG sites in the *INS* gene with disease progression might limit the sensitivity of this sequencing-based approach.

To date, most studies employing differentially methylated DNA-based biomarker assays have focused on the *INS* gene, primarily due to the specific expression of the gene in β-cells. The potential for other β-cell-specific genes (e.g., *IAPP*) was recently demonstrated [38]. With emerging data on whole genome methylation sequencing, it should be possible to identify new, perhaps more specific, methylated and unmethylated CpGs in β-cells in an unbiased manner without consideration of gene expression patterns.

Conclusions and Future Directions

The identification of robust panels of β-cell-specific biomarkers is a field still in its infancy. Nevertheless, the observations on PI/C ratios and unmethylated *INS* DNA in at-risk cohorts [27••, 37••] are suggestive of the potential for these and other biomarkers to identify populations of subjects who are more likely to develop T1D. Hurdles that remain include (a) persistent issues regarding sensitivity and specificity of biomarkers that reflect both the need to identify very low-level β-cell stress/death and to ensure that the signals are emanating from β-cells and not other uninvolved cell types, and (b) miniaturization and standardization of assays such that minimal blood volumes are required and that assays can be performed in multiple laboratories, (c) biomarker half-life considerations as β stress/death may be cyclical during stages 1 and 2, and (d) careful consideration of the heterogeneity of T1D with the acknowledgement that important differences in disease progression are driven by age and underlying genetic risk. Some of these hurdles, particularly specificity, can be overcome by utilizing a panel of biomarkers that collectively can be used to derive a score for β-cell health. Additionally, given the growth of “omics” data, the possibility that other as yet unidentified proteins (e.g., proteins emanating from alternatively spliced mRNAs) and molecular species (e.g., lipid and metabolite species) uniquely emanating from β-cells during the process of T1D development has yet to be explored.

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References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Lehuen A, Diana J, Zaccone P, Cooke A. Immune cell crosstalk in type 1 diabetes. Nat Rev Immunol. 2010; 10:501–13. [PubMed: 20577267]
2. Herold KC, Gitelman SE, Masharani U, Hagopian W, Bisikirska B, Donaldson D, et al. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. Diabetes. 2005; 54:1763–9. [PubMed: 15919798]

3. Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med. 2002; 346:1692–8. [PubMed: 12037148]
4. Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet. 2011; 378:412–9. [PubMed: 21719096]
5. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med. 2009; 361:2143–52. [PubMed: 19940299]
6. Rigby MR, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Diab Endocrinol. 2013; 1:284–94.
7. Gregg BE, Moore PC, Demozay D, Hall BA, Li M, Husain A, et al. Formation of a human β-cell population within pancreatic islets is set early in life. J Clin Endocrinol Metab. 2012; 97:3197–206. [PubMed: 22745242]
8. Meier JJ, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. Diabetes. 2008; 57:1584–94. [PubMed: 18334605]
9. Atkinson MA, Bluestone JA, Eisenbarth GS, Hebrok M, Herold KC, Accili D, et al. How does type 1 diabetes develop?: the notion of homicide or β-cell suicide revisited. Diabetes. 2011; 60:1370–9. [PubMed: 21525508]
10. Maganti A, Evans-Molina C, Mirmira R. From immunobiology to β-cell biology: the changing perspective on type 1 diabetes. Islets. 2014; 6:e28778. [PubMed: 25483958]
11. Marré ML, James EA, Piganelli JD. β cell ER stress and the implications for immunogenicity in type 1 diabetes. Front Cell Dev Biol. 2015; 3:67. [PubMed: 26579520]
12. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the endocrine society, and the American Diabetes Association. Diabetes Care. 2015; 38:1964–74. [PubMed: 26404926]
13. Butler AE, Cao-Minh L, Galasso R, Rizza RA, Corradin A, Cobelli C, et al. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. Diabetologia. 2010; 53:2167–76. [PubMed: 20523966]
14. Eizirik DL, Miani M, Cardozo AK. Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia. 2013; 56:234–41. [PubMed: 23132339]
15. Engin F, Yermalovich A, Nguyen T, Hummasti S, Fu W, Eizirik DL, et al. Restoration of the unfolded protein response in pancreatic beta cells protects mice against type 1 diabetes. Sci Transl Med. 2013; 5:211ra156.
16. Tersey SA, Nishiki Y, Templin AT, Cabrera SM, Stull ND, Colvin SC, et al. Islet β-cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. Diabetes. 2012; 61:818–27. [PubMed: 22442300]
17. Yang C, Diiorio P, Jurczyk A, O'Sullivan-Murphy B, Urano F, Bortell R. Pathological endoplasmic reticulum stress mediated by the IRE1 pathway contributes to pre-insulitic beta cell apoptosis in a virus-induced rat model of type 1 diabetes. Diabetologia. 2013; 56:2638–46. [PubMed: 24121653]
18. Marhfour I, Lopez XM, Lefkaditis D, Salmon I, Allagnat F, Richardson SJ, et al. Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. Diabetologia. 2012; 55:2417–20. [PubMed: 22699564]
19. Liu M, Wright J, Guo H, Xiong Y, Arvan P. Proinsulin entry and transit through the endoplasmic reticulum in pancreatic beta cells. Vitam Horm. 2014; 95:35–62. [PubMed: 24559913]
20. Engin F. ER stress and development of type 1 diabetes. J Investig Med. 2016; 64:2–6.
21. Ludvigsson J, Heding L. Abnormal proinsulin/C-peptide ratio in juvenile diabetes. Acta Diabetol Lat. 1982; 19:351–8. [PubMed: 6758459]

22. Snorgaard O, Hartling SG, Binder C. Proinsulin and C-peptide at onset and during 12 months cyclosporin treatment of type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1990; 33:36–42. [PubMed: 2303172]

23. Watkins RA, Evans-Molina C, Terrell JK, Day KH, Guindon L, Restrepo IA, et al. Proinsulin and heat shock protein 90 as biomarkers of beta-cell stress in the early period after onset of type 1 diabetes. Transl Res. 2016; 168:96–106. e1. [PubMed: 26397425]

24. Schölin A, Nyström L, Arnqvist H, Bolinder J, Björk E, Berne C, et al. Proinsulin/C-peptide ratio, glucagon and remission in new-onset type 1 diabetes mellitus in young adults. Diabet Med. 2011; 28:156–61. [PubMed: 21219422]

25. Røder ME, Knip M, Hartling SG, Karjalainen J, Akerblom HK, Binder C. Disproportionately elevated proinsulin levels precede the onset of insulin-dependent diabetes mellitus in siblings with low first phase insulin responses. The Childhood Diabetes in Finland Study Group. J Clin Endocrinol Metab. 1994; 79:1570–5. [PubMed: 7989457]

26. Truyen I, De Pauw P, Jørgensen PN, Van Schravendijk C, Ubani O, Decochez K, et al. Proinsulin levels and the proinsulin:C-peptide ratio complement autoantibody measurement for predicting type 1 diabetes. Diabetologia. 2005; 48:2322–9. [PubMed: 16211374]

27••. Sims EK, Chaudhry Z, Watkins RA, Syed F, Blum J, Fangqian O, et al. Elevations in the fasting serum proinsulin:C-peptide ratio precede the onset of type 1 diabetes. Diabetes Care. 2016; Sims and colleagues demonstrated an increase in the PI:C ratio 12 months prior to T1D diagnosis in subjects followed in the TrialNet Pathway to Prevention study, with the most pronounced elevations observed in subjects ≤10 years of age. Logistic regression analysis, adjusted for age and body mass index, demonstrated an increased odds of progression to T1D with higher PI:C ratios. doi: 10.2337/dc15-2849

28. Erener S, Mojibian M, Fox JK, Denroche HC, Kieffer TJ. Circulating miR-375 as a biomarker of β-cell death and diabetes in mice. Endocrinology. 2013; 154:603–8. [PubMed: 23321698]

29••. Kanak MA, Takita M, Shahbazov R, Lawrence MC, Chung WY, Dennison AR, et al. Evaluation of MicroRNA375 as a novel biomarker for graft damage in clinical islet transplantation. Transplantation. 2015; 99:1568–73. Circulating levels of miR-375 in persons undergoing autologous or allogeneic islet transplantation were analyzed and plasma miR-375 levels were found to be significantly higher in recipients of islet transplants compared to a control group who had not undergone transplantation. [PubMed: 25769078]

30. Roggli E, Britan A, Gattesco S, Lin-Marq N, Abderrahmani A, Meda P, et al. Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic beta-cells. Diabetes. 2010; 59:978–86. [PubMed: 20086228]

31. Nielsen LB, Wang C, Sørensen K, Bang-Berthelsen CH, Hansen L, Andersen M-LM, et al. Circulating levels of microRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that miR-25 associates to residual beta-cell function and glycaemic control during disease progression. Exp Diabetes Res. 2012; 2012:896362. [PubMed: 22829805]

32. Kim KW, Ho A, Alshabee-Akil A, Hardikar AA, Kay TWH, Rawlinson WD, et al. Coxsackievirus B5 infection induces dysregulation of microRNAs predicted to target known type 1 diabetes risk genes in human pancreatic islets. Diabetes. 2016; 65:996–1003. [PubMed: 26558682]

33. Husseiny MI, Kaye A, Zebadua E, Kandeel F, Ferreri K. Tissue-specific methylation of human insulin gene and PCR assay for monitoring beta cell death. PLoS ONE. 2014; 9:e94591. [PubMed: 24722187]

34••. Lehmann-Werman R, Neiman D, Zemmour H, Moss J, Magenheim J, Vaknin-Dembinsky A, et al. Identification of tissue-specific cell death using methylation patterns of circulating DNA. Proc Natl Acad Sci U S A. 2016; 113:E1826–34. In this article, Lehmann-Werman and colleagues introduced a cell-free insulin DNA assay based on detection of the methylation status of 6 regional CpG sites in the insulin promoter. The authors found that de-methylation at all 6 sites was present in ~80% of DNA molecules from β cells compared to less than 0.01% of DNA from other tissues. The assay was subsequently validated in samples from subjects with T1D and in persons undergoing islet transplantation. [PubMed: 26976580]

35. Akirav EM, Lebastchi J, Galvan EM, Henegariu O, Akirav M, Ablamunits V, et al. Detection of β cell death in diabetes using differentially methylated circulating DNA. Proc Natl Acad Sci U S A. 2011; 108:19018–23. [PubMed: 22074781]

36••. Fisher MM, Watkins RA, Blum J, Evans-Molina C, Chalasani N, DiMeglio LA, et al. Elevations in circulating methylated and unmethylated preproinsulin DNA in new-onset type 1 diabetes. Diabetes. 2015; 64:3867–72. Utilizing a droplet digital PCR-based assay, Fisher and colleagues demonstrated that circulating levels of both unmethylated and methylated insulin DNA were elevated in subjects with new onset T1D. Methylated insulin DNA remained elevated up to 8 weeks after T1D onset, while unmethylated insulin DNA levels decreased after diagnosis. [PubMed: 26216854]

37••. Herold KC, Usmani-Brown S, Ghazi T, Lebastchi J, Beam CA, Bellin MD, et al. β cell death and dysfunction during type 1 diabetes development in at-risk individuals. J Clin Invest. 2015; 125:1163–73. Herold and colleagues analyzed samples from the TrialNet Pathway to Prevention Cohort and found that individuals at high risk for T1D had increased levels of unmethylated insulin DNA during the presymptomatic phase of T1D. [PubMed: 25642774]

38. Olsen JA, Kenna LA, Spelios MG, Hessner MJ, Akirav EM. Circulating differentially methylated amylin DNA as a biomarker of β-cell loss in type 1 diabetes. PLoS ONE. 2016; 11:e0152662. [PubMed: 27111653]

39. Hartling SG, Knip M, Røder ME, Dinesen B, Akerblom HK, Binder C. Longitudinal study of fasting proinsulin in 148 siblings of patients with insulin-dependent diabetes mellitus. Study Group on Childhood Diabetes in Finland. Eur J Endocrinol. 1997; 137:490–4. [PubMed: 9405028]

40. Heding LG, Ludvigsson J. Human proinsulin in insulin-treated juvenile diabetics. Acta Paediatr Scand Suppl. 1977:48–52. [PubMed: 278468]

41. Hartling SG, Lindgren F, Dahlqvist G, Persson B, Binder C. Elevated proinsulin in healthy siblings of IDDM patients independent of HLA identity. Diabetes. 1989; 38:1271–1274. [PubMed: 2792577]

42. Lindgren FA, Hartling SG, Dahlquist GG, Binder C, Efendic S, Persson BE. Glucose-induced insulin response is reduced and proinsulin response increased in healthy siblings of type 1 diabetic patients. Diabet Med. 1991; 8:638–643. [PubMed: 1680601]

43. Spinas GA, Snorgaard O, Hartling SG, Oberholzer M, Berger W. Elevated proinsulin levels related to islet cell antibodies in first-degree relatives of IDDM patients. Diabetes Care. 1992; 15:632–637. [PubMed: 1516482]

44. Heaton DA, Millward BA, Gray P, Tun Y, Hales CN, Pyke DA, Leslie RD. Evidence of beta cell dysfunction which does not lead on to diabetes: a study of identical twins of insulin dependent diabetics. Br Med J (Clin Res Ed). 1987; 294:145–146.

45. Heaton DA, Millward BA, Gray IP, Tun Y, Hales CN, Pyke DA, Leslie RD. Increased proinsulin levels as an early indicator of B-cell dysfunction in non-diabetic twins of type 1 (insulin-dependent) diabetic patients. Diabetologia. 1988; 31:182–184. [PubMed: 3286344]

46. Lindgren FA, Hartling SG, Persson BE, Roder ME, Snellman K, Binder C, Dahlquist G. Proinsulin levels in newborn siblings of type 1 (insulin-dependent) diabetic children and their mothers. Diabetologia. 1993; 36:560–563. [PubMed: 8335179]

47. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001; 294:853–8. [PubMed: 11679670]

48. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science. 2001; 294:858–62. [PubMed: 11679671]

49. Ørom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5′UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell. 2008; 30:460–71. [PubMed: 18498749]

50. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science. 2007; 318:1931–4. [PubMed: 18048652]

51. miRBase [Internet]. [cited 2016 May 8]. Available from: http://www.mirbase.org/

52. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014; 42:D68–73. [PubMed: 24275495]

53. Guay C, Menoud V, Rome S, Regazzi R. Horizontal transfer of exosomal microRNAs transduce apoptotic signals between pancreatic beta-cells. Cell Commun Signal. 2015; 13:17. [PubMed: 25880779]

54. Lakhter AJ, Sims EK. Minireview: emerging roles for extracellular vesicles in diabetes and related metabolic disorders. Mol Endocrinol. 2015; 29:1535–48. [PubMed: 26393296]

55. Lee Y, El Andaloussi S, Wood MJA. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet. 2012; 21:R125–34. [PubMed: 22872698]
56. Barutta F, Tricarico M, Corbelli A, Annaratone L, Pinach S, Grimaldi S, et al. Urinary exosomal microRNAs in incipient diabetic nephropathy. PLoS One. 2013; 8:e73798. [PubMed: 24223694]
57. Bijkerk R, Duijs JMGJ, Khairoun M, Ter Horst CJH, van der Pol P, Mallat MJ, et al. Circulating microRNAs associate with diabetic nephropathy and systemic microvascular damage and normalize after simultaneous pancreas-kidney transplantation. Am J Transplant. 2015; 15:1081–90. [PubMed: 25716422]
58. Figliolini F, Cantaluppi V, Lena MD, Beltramo S, Romagnoli R, Salizzoni M, et al. Isolation, characterization and potential role in beta cell-endothelium cross-talk of extracellular vesicles released from human pancreatic islets. PLoS ONE. 2014; 9:e102521. [PubMed: 25028931]
59. de la Torre García N, Fernández-Durango R, Gómez R, Fuentes M, Roldán-Pallarés M, Donate J, et al. Expression of angiogenic microRNAs in endothelial progenitor cells from type 1 diabetic patients with and without diabetic retinopathy. Invest Ophthalmol Vis Sci. 2015; 56:4090–8. [PubMed: 26114486]
60. Guay C, Regazzi R. Circulating microRNAs as novel biomarkers for diabetes mellitus. Nat Rev Endocrinol. 2013; 9:513–21. [PubMed: 23629540]
61. Zampetaki A, Willeit P, Burr S, Yin X, Langley SR, Kiechl S, et al. Angiogenic microRNAs linked to incidence and progression of diabetic retinopathy in type 1 diabetes. Diabetes. 2016; 65:216–27. [PubMed: 26395742]
62. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clin Chem. 2010; 56:1733–41. [PubMed: 20847327]
63. van de Bunt M, Gaulton KJ, Parts L, Moran I, Johnson PR, Lindgren CM, et al. The miRNA profile of human pancreatic islets and beta-cells and relationship to type 2 diabetes pathogenesis. PLoS ONE. 2013; 8:e55272. [PubMed: 23372846]
64. Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, et al. A pancreatic islet-specific microRNA regulates insulin secretion. Nature. 2004; 432:226–30. [PubMed: 15538371]
65. Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, et al. miR-375 maintains normal pancreatic alpha- and beta-cell mass. Proc Natl Acad Sci U S A. 2009; 106:5813–8. [PubMed: 19289822]
66••. Latreille M, Herrmanns K, Renwick N, Tuschl T, Malecki MT, McCarthy MI, et al. miR-375 gene dosage in pancreatic β-cells: implications for regulation of β-cell mass and biomarker development. J Mol Med. 2015; 93:1159–69. This article found that only a relatively small proportion (~1%) of circulating miR-375 originates from β cells, suggesting utility of this miRNA as a biomarker of β cell death but not β cell mass. [PubMed: 26013143]
67. Osipova J, Fischer D-C, Dangwal S, Volkmann I, Widera C, Schwarz K, et al. Diabetes-associated microRNAs in pediatric patients with type 1 diabetes mellitus: a cross-sectional cohort study. J Clin Endocrinol Metab. 2014; 99:E1661–5. [PubMed: 24937532]
68. Sebastiani G, Grieco FA, Spagnuolo I, Galleri L, Cataldo D, Dotta F. Increased expression of microRNA miR-326 in type 1 diabetic patients with ongoing islet autoimmunity. Diabetes Metab Res Rev. 2011; 27:862–6. [PubMed: 22069274]
69. Wu G-C, Pan H-F, Leng R-X, Wang D-G, Li X-P, Li X-M, et al. Emerging role of long noncoding RNAs in autoimmune diseases. Autoimmun Rev. 2015; 14:798–805. [PubMed: 25989481]
70. NONCODE [Internet]. [cited 2016 May 14]. Available from: http://www.noncode.org/
71. Hakonarson H, Grant SFA, Bradfield JP, Marchand L, Kim CE, Glessner JT, et al. A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. Nature. 2007; 448:591–4. [PubMed: 17632545]
72. Plagnol V, Howson JMM, Smyth DJ, Walker N, Hafler JP, Wallace C, et al. Genome-wide association analysis of autoantibody positivity in type 1 diabetes cases. PLoS Genet. 2011; 7:e1002216. [PubMed: 21829393]
73. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet. 2007; 39:857–64. [PubMed: 17554260]

74. Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG. The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. Nat Genet. 2010; 42:68–71. [PubMed: 19966805]

75. Motterle A, Gattesco S, Caille D, Meda P, Regazzi R. Involvement of long non-coding RNAs in beta cell failure at the onset of type 1 diabetes in NOD mice. Diabetologia. 2015; 58:1827–35. [PubMed: 26037202]

76. Carter G, Miladinovic B, Patel AA, Deland L, Mastorides S, Patel NA. Circulating long noncoding RNA GAS5 levels are correlated to prevalence of type 2 diabetes mellitus. BBA Clin. 2015; 4:102–7. [PubMed: 26675493]

77. Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011; 11:426–37. [PubMed: 21562580]

78. Schübeler D. Function and information content of DNA methylation. Nature. 2015; 517:321–6. [PubMed: 25592537]

79. Stebbing J, Bower M, Syed N, Smith P, Yu V, Crook T. Epigenetics: an emerging technology in the diagnosis and treatment of cancer. Pharmacogenomics. 2006; 7:747–57. [PubMed: 16886899]

80. Grady WM, Rajput A, Lutterbaugh JD, Markowitz SD. Detection of aberrantly methylated hMLH1 promoter DNA in the serum of patients with microsatellite unstable colon cancer. Cancer Res. 2001; 61:900–2. [PubMed: 11221878]

81. Kuroda A, Rauch TA, Todorov I, Ku HT, Al-Abdullah IH, Kandeel F, et al. Insulin gene expression is regulated by DNA methylation. PLoS One. 2009; 4:e6953. [PubMed: 19742322]

82. Yang BT, Dayeh TA, Kirkpatrick CL, Taneera J, Kumar R, Groop L, et al. Insulin promoter DNA methylation correlates negatively with insulin gene expression and positively with HbA1c levels in human pancreatic islets. Diabetologia. 2010; 54:360–7. [PubMed: 21104225]

83. Husseiny MI, Kuroda A, Kaye AN, Nair I, Kandeel F, Ferreri K. Development of a quantitative methylation-specific polymerase chain reaction method for monitoring beta cell death in type 1 diabetes. PLoS One. 2012; 7:e47942. [PubMed: 23144715]

84. Lebastchi J, Deng S, Lebastchi AH, Beshar I, Gitelman S, Willi S, et al. Immune therapy and β-cell death in type 1 diabetes. Diabetes. 2013; 62:1676–80. [PubMed: 23423576]

85. Fisher MM, Perez Chumbiauca CN, Mather KJ, Mirmira RG, Tersey SA. Detection of islet β-cell death in vivo by multiplex PCR analysis of differentially methylated DNA. Endocrinology. 2013; 154:3476–81. [PubMed: 23825129]

86. Usmani-Brown S, Lebastchi J, Steck AK, Beam C, Herold KC, Ledizet M. Analysis of β-cell death in type 1 diabetes by droplet digital PCR. Endocrinology. 2014; 155:3694–8. [PubMed: 25004096]

87•. Rui J, Deng S, Lebastchi J, Clark PL, Usmani-Brown S, Herold KC. Methylation of insulin DNA in response to proinflammatory cytokines during the progression of autoimmune diabetes in NOD mice. Diabetologia. 2016; 59:1021–9. The authors showed that during progression to Type 1 diabetes, the methylation status of the insulin gene in islets from NOD mice changed in a dynamic fashion. The authors also demonstrated that treatment of isolated islets with a cocktail of pro-inflammatory cytokines led to increased methylation of the insulin gene. [PubMed: 26910463]

Table 1

Potential biomarkers of β-cell stress and death in type 1 diabetes

Potential biomarker	Data from ex vivo or preclinical T1D models	Human data	
Proinsulin/C-peptide (PI/C) Ratio	Proinsulin/insulin ratios were increased in pre-diabetic and diabetic NOD mice [16].	**1**	PI/C ratios were increased in recent-onset T1D and reductions in the PI/C ratio were associated with T1D remission [21–24].
		2	PI/C ratios were increased in autoantibody-positive individuals who progressed to T1D [25, 26, 27••].
		3	Elevated PI/C ratios were found to be predictive of T1D onset [27••].
miR-375	Increased in plasma of streptozotocin-treated and pre-diabetic NOD mice [28].	Elevated in sera after allogeneic and autologous islet transplantation [29••].	
miR-21, miR-34a, and miR-146a	Increased in human islets treated with proinflammatory cytokines and in islets of pre-diabetic NOD mice [30].		
miR-152, miR-30a-5p, miR-181a, miR-24, miR-148a, miR-210, miR-27a, miR-29a, miR-26a, miR-27b, miR-25, and miR-200a		Increased in sera of humans with recent-onset T1D [31].	
miR-34a-5p, miR-21-3p, miR155-5p, miR-1290, miR663b, miR10b-3p	Increased in human islets infected with Coxsackievirus B5 [32].		
Unmethylated *INS* DNA		**1**	Increased in humans undergoing allogeneic islet transplantation [33, 34••].
		2	Increased in recent-onset T1D [34••, 35, 36••].
		3	Increased in high-risk autoantibody-positive individuals prior to the onset of T1D [37••].
Unmethylated *IAPP* DNA	Increased in sera of pre-diabetic and diabetic NOD mice [38].	Increased in recent-onset T1D [38].	